CHEMICAL AND PHYSICAL CHANGES IN GEOTROPIC STIMULATION AND RESPONSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 179

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(WITH SIX FIGURES)

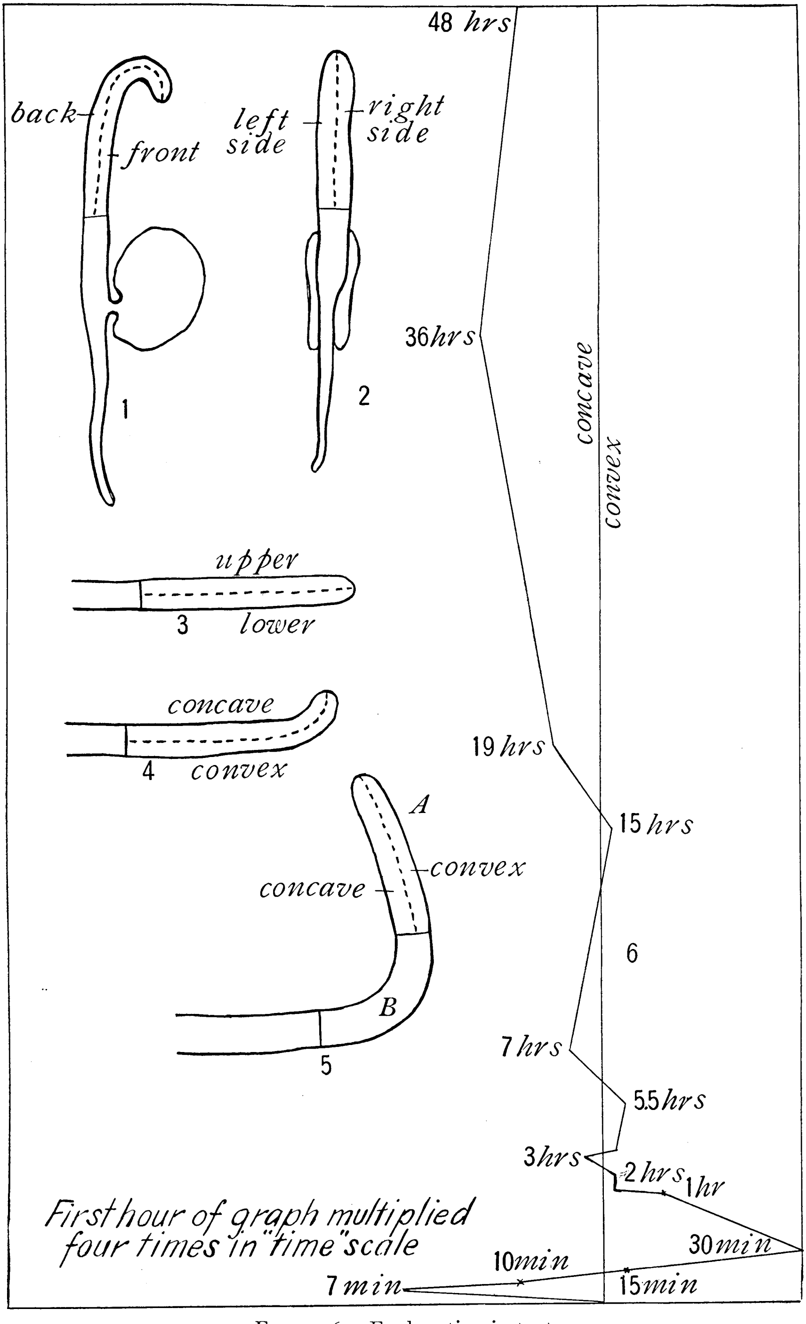
Considerable work has been done on chemical-physical changes involved in tropic presentation and reaction. KRAUS (1) was probably the first worker in this field. His researches include the determination of (*a*) the sugar content of the growing shoot, (*b*) the relation of the sugar maximum to the growth maximum, (*c*) acidity in the growing shoot; and (*d*) steps in the change of the cell-content of the two halves (the shoot was split longitudinally into the concave half, the inside of the curved portion, and convex half, the outside of the curved portion, of the responded organ) of the shoot exposed to geotropic or heliotropic stimuli during perception and reaction time. He found that (*a*) the sugar in the growing shoot increases for a certain region from above downward and then decreases, (*b*) the sugar maximum lies below the growth maximum and consequently is not the limiting factor in growth, (*c*) the acidity is greatest in the tip and decreases downward. In geotropically and heliotropically stimulated shoots he found, first, decreased acidity and increased sugar on the convex side, later, an increase of water and decrease of sugar on the convex side, followed by a decrease of acidity on this side.

CZAPEK (2) working with geotropically stimulated roots found an interference with the oxidation of tyrosin and phenylalanine which led to the production of homogentisic acid. GROTTIAN (3) and GRAFE and LINSBAUER (4) were unable to confirm his results. MARTIN FISCHER (5) has shown that the water-absorption power of colloids is increased by the addition of acids and alkalies up to a certain concentration, and that salts decrease this power. PROMSY (6) has found that organic acids increase the growth rate of seedlings. RAVIN (7) likewise found that acids increase the rate of

elongation in growing plant organs. ECKERSON (8) has shown that acids decrease the period of after-ripening of certain dormant seeds. The activity of these acids on germination is due in part to the transformation of zymogens into active enzymes and to favoring the activity of those enzymes. In both germination and growth it is probably partly due to increasing the water-absorbing power of the colloids, especially of the protoplasm.

In view of the effect of acidity on the absorption of water and on the growth rate, it was thought best to reexamine the two halves (concave and convex) of geotropically stimulated and responding organs for difference in acidity, and incidentally for other features such as the sugar content. Following FISCHER's suggestion, it was thought that relative growth on the two sides might parallel the acidity. A later work will examine into this condition of tropic and nastic responses, and a later paper will relate the data more fully to the literature.

Etiolated seedlings grown upright on boards under a spray have been used throughout the experiment. The temperature maintained was approximately 16° C. When the seedlings were 6–8 cm. high, they were used in making tests of acidity upon either the unstimulated or the stimulated organ. For the latter tests the board containing the seedlings was turned on the side so that the seedlings were brought at right angles to gravity. Tests for acidity were made at various intervals of presentation and reaction time, ranging from 7 minutes to 48 hours. In making the tests on the unstimulated shoots, the vertical stem was split longitudinally into right and left halves (in the plane of the cotyledons), and also into back and front halves (at right angles to the plane of the cotyledons) (figs. 1 and 2). For the tests on the geotropically stimulated seedlings the shoots were split longitudinally into upper and lower halves, the former being the half away from the direction of the stimulus and the latter the half next the direction of the stimulus. After reaction these showed as concave and convex halves respectively. These terms hold throughout the paper (figs. 3 and 4). The terminal 4.5 cm. was used, since this region included the region of curvature and since tests on acidity in successive centimeters from the tip downward showed the maximum acidity to be included



FIGS. 1–6.—Explanation in text

in this portion. The cut seedlings were weighed in weighing-bottles and the weight obtained by difference. Samples of 6–8 gm. were used.

Immediately after weighing, the samples were cut up fine and ground to a pulp in a mortar. Several methods of titration were used. In the first method employed, about 50 cc. of distilled water were added to the ground-up tissue and the sample titrated at once without filtration, using phenolphthalein or neutral red as indicator. The objection to the method is that reaction between the acid yet in the tissue with the alkali is slow yet continuous, so that the end point is not definite. A second method used was the test plate method. The tissue was ground as before, filtered through glass wool, and the filtrate refiltered through asbestos over a filter pump. The tissue together with the glass wool filter was again triturated and washed quantitatively into the filtrate previously obtained and the volume made up to 100 cc.; 10 or 25 cc. were used in a titration and the average of several titrations used in calculation. The end point was determined by means of phenolphthalein in the following way. Two solutions of phenolphthalein were prepared. The first was prepared by adding 10 drops of an alcoholic solution of phenolphthalein to 25 cc. of distilled water. The second solution contained in addition 3 drops of a $n/20$ solution of NaOH, enough to make the solution a decided pink color. A few drops of each solution were placed on a test plate and the sample being titrated tested by introducing a small quantity on the end of a small stirring rod into each of the solutions of phenolphthalein. The end point was reached when the sample introduced just failed to neutralize the small amount of alkali in the one solution, as indicated by the faint pink color remaining, and just showed a faint pink tinge in the other solution. In this way the end point was not obscured by the color of the solution. The method finally adopted was that of using the natural indicator in the plant itself. It has been observed in the course of both animal and plant tissue analysis that chromogens develop when certain fractions, as the lipoid fraction, are neutralized. This suggested the idea that possibly the raw material upon neutralization might show a change of color which could be used as an indicator. The reliability of the natural

indicator was tested on several different plants. The results of the experiment are given elsewhere in this paper.

In the case of *Vicia Faba* the material was prepared as in the test plate method; 10–25 cc. of the solution were placed in a porcelain dish and titrated with a $n/10$ solution of sodium hydroxide. The solution was at first grayish opalescent. It gradually changed during titration, assuming a yellowish color. Upon neutralization the solution turned the color of the testa of the ripe *Vicia Faba* seed. Excess of sodium hydroxide does not change the color. Upon standing 15–30 minutes, however, the neutralized solution becomes dark brown.

The work on acidity included (*a*) tests on the region of greatest acidity in the shoot, (*b*) tests on the right and left halves and back and front halves of the unstimulated shoot, and (*c*) tests on the upper and lower (concave and convex) halves of the geotropically stimulated shoots. The following table will give illustrations of the data obtained in experiments *a* and *b*. The first table gives

TABLE I

Vicia Faba shoots in 0.5 cm. lengths from tip downward

1st 0.5 cm	1.30 cc. per g. wt.
2d 0.5 cm	1.25 cc. " " "
3d 0.5 cm	1.07 cc. " " "
4th 0.5 cm	0.84 cc. " " "

TABLE II

Vicia Faba SHOOTS; TERM. 4.5 CM. USED

Right half	Left half	Front	Back
0.77	0.71	0.91	0.92
0.88	0.88	0.84	0.83
0.89	0.89	0.98	0.94
0.70	0.67	0.77	0.78
Av. 0.80	0.78	0.87	0.87

results for tests on acidity in 0.5 cm. lengths from the tip downward. The second table shows the acidity of the unstimulated shoots cut longitudinally into right and left halves and front and back halves (figs. 1 and 2). The results as given in table I show decreased acidity from the tip downward, which accords with the work of

Kraus. Table II is given merely to show the probable error in titration in the following acidity tests of the stimulated shoots.

Table III shows results of titration of the geotropically stimulated shoots at varying intervals of time corresponding to the periods of maximum acidity and to the period of equal acidity of the concave and convex halves.

TABLE III

Vicia Faba SHOOTS GEOTROPICALLY STIMULATED; TERMINAL 4.5 CM. USED
NO. CC. PER GRAM FRESH WT.

Time stim.	Concave half	Convex half	Diff. in acidity
7 minutes	0.942	0.715	0.237
10 "	0.622	0.469	0.153
15 "	0.623	0.683	0.060
30 "	0.900	1.210	0.300
60 "	• 1.07	1.19	0.13
2 hours	1.01	1.02	0.01
17 "	0.512	0.515	0.03
38 "	0.884	0.782	0.102
48 "	0.671	0.589	0.072

From the tables it will be noticed that the geotropically stimulated shoot first increases in acidity in the upper (concave) half, the observed maximum being 7 minutes. After the maximum the acidity of the concave side rapidly diminishes, passing a period of about equal acidity in 15 minutes. The convex side more slowly approaches a maximum acidity, reaching it in 30 minutes. After the maximum the two sides gradually become equal in acidity until at the time of visible response, about two hours, they are practically equal. This equality in acidity continues through the period of curvature till the plant has passed the vertical, when the concave side again becomes more acid (for these titrations only the curved portion which had passed the vertical plane were used; fig. 5, *A*). As the shoot straightens again, the acidity decreases on the concave side. The accompanying graph (fig. 6) gives the average of several experiments for each time indicated.

Since this method measures the titration value but not the H-ion content of the acid, the results are not directly comparable with the work of Fischer, whose conception, besides the acid change, involves also changes in the amount of salts and the nature

of the colloids, which have not been undertaken in this work. So far as the results indicate, however, they show no difference which would explain curvature on the basis of increased acidity, since at the time visible curvature begins the two flanks are of equal acidity.

In testing the natural indicator of the plant, the tissue was prepared as described above for *Vicia Faba;* 25 cc. of the filtrate were used in titration and several duplicate titrations made. Table IV serves to show the accuracy of the titration and to give the color reaction.

TABLE IV

Tissue used	No. cc. *n*/10 NaOH to neut. 25 cc. sol.			Color unneut. sol.	Color neut. sol.
Mucor (young)	0.13	0.13	0.12	Faintly greenish	Color ripe spores
Wax beans	0.36	0.36	0.40	Opalescent	Pale canary yellow
Ripe apple	1.61	1.65	1.60	Pale cider	Dark orange
Lemon juice	14.80	14.75	14.75	Lemonade	Color lemon rind
Lemon rind	0.26	0.23	0.28	Lemon rind	Orange rind
Orange juice	2.12	2.18	2.14	Dilute orange juice	Orange rind
Orange rind	0.29	0.30	0.30	Pale orange	Deep orange
Etiolated seedlings—					
Corn	0.46	0.46	0.44	Faintly yellow	Orange yellow
Sweet peas	0.40	0.41	0.40	Opalescent	Canary yellow
Sunflower	0.23	0.23	0.20	Dull brownish yellow	Bright greenish yellow
Vicia Faba	0.71	0.72	0.70	Faintly grayish	Color ripe seed

Neutral red and phenolphthalein were used with the test plate as checks in determining the accuracy of the indicator. The neutral point is reached when a drop of the alkali fails to produce a change of color in the solution. It is interesting to note that the color produced in neutralization is usually the color of some part of the seed or plant. In *Vicia Faba* this similarity is striking. Solutions of the triturated coat are neutral. The chromogens produced in all of the solutions neutralized were precipitated in 24–48 hours.

Only one sugar titration has been made. For this analysis the etiolated seedlings were grown in sand in the greenhouse at a temperature of about 16° C. When the seedlings were 6–8 cm. high, they were geotropically stimulated for 30 minutes and the terminal 4.5 cm. used in the analysis. The gathering required

half an hour, so that the average stimulation was 45 minutes. After weighing, the two halves (upper and lower) were placed in 85 per cent alcohol heated to 70° C., and allowed to stand for two weeks. The material was then cut up fine and extracted 48 hours in an extractor similar to the Soxlet extractor. The extract together with the alcohol used in preserving the material was evaporated to moist dryness over the steam bath and brought to constant weight *in vacuo*. The dried material was dissolved in water, 10 cc. of concentrated hydrochloric acid and 20 cc. of chloroform added, and the whole made up to 1000 cc. in volume. The water-soluble portion was filtered from the chloroform-soluble or lipoid fraction and the sugar determination made upon it directly. The lipoid fraction was dissolved in alcohol, and made up to 500 cc. in volume; 50 cc. of each fraction was used in the determination. The fractions were hydrolized and the tannin precipitated before testing for sugar. The method of hydrolysis used was that described in *Bulletin no. 107*, p. 41, U.S. Dept. of Agric., Bur. of Chem. The tannin was precipitated after hydrolysis by means of lead acetate and sodium sulphate. The sugar was determined by the cuprous oxide method as described in the above mentioned bulletin (p. 242). The amount of cuprous oxide was determined by the volumetric potassium permanganate method (*ibid.* pp. 52, 53). An $n/20$ solution was used. The calculations were based on the mg. of copper oxidized in the change from cuprous to cupric oxide, and the invert sugar equivalent obtained from the accompanying table (p. 243). Table V shows the results obtained.

TABLE V

Flank	Fresh wt.	Dry wt.	Amt. sugar	Percentage fresh wt.	Percentage dry wt.
Concave.....	231.635	11.2994	2.7763	1.198	24.50
Convex	242.275	11.8064	1.9202	0.792	16.26

These results differ from those of KRAUS in point of time of stimulation. He found the sugar content on the convex side to increase during the period of one hour and then to decrease. The experiment given shows an increase of 0.406 per cent of the fresh

weight and 8.24 per cent of the dry weight on the concave side of seedlings stimulated 45 minutes.

The dry weight of the curved portion of the shoot (fig. 5, *B*) was determined for various times of stimulation. The material was split into concave and convex halves, weighed, and dried to constant weight in an electric oven at a temperature of 104° C. The differences obtained, while slight, are significant in that they vary in the same direction. Table VI explains itself.

TABLE VI

Time stim. days	Fresh wt. concave grams	Fresh wt. convex grams	Dry wt. concave grams	Dry wt. convex grams	Percentage dry wt. concave	Percentage dry wt. convex	Diff.
2.5.....	22.0139	26.6482	1.2480	1.4234	5.44	5.34	0.09
3.0.....	17.0296	20.8236	.9202	1.0746	5.43	5.16	0.17
4.0.....	54.1735	62.2810	2.8621	3.1524	5.20	5.06	0.14

Summary

1. The acidity of the growing shoot is greatest at the tip and decreases downward.

2. The relative acidity of the two flanks of the geotropically stimulated shoots changes during presentation and reaction time. First the concave side becomes relatively more acid, then decreases until the maximum acidity comes to lie on the convex side. The two flanks now gradually become equal in acidity, this period coinciding with the time of visible curvature. This equality in acidity is maintained until the tip of the shoot has passed the vertical plane, when the concave side again becomes more acid. As the shoot straightens, the difference in acidity decreases.

3. The increase of acidity does not parallel the relative rate of growth on the two flanks.

4. Several plants examined develop in neutral solution a chromogen which acts as a delicate acid-alkali indicator.

5. The percentage of dry weight is greatest on the concave side.

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